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ICH Considerations

General Principles to Address the Risk of Inadvertent Germline Integration of Gene Therapy Vectors

1. Introduction

Gene therapy vectors are designed to introduce genetic material into patients' cells for therapeutic, preventive or diagnostic purposes. Integration of delivered gene(s) into the genome of target cells represents the therapeutic goal in some clinical applications, for example when long-term efficacy/therapy is sought in genetic diseases. Also, vectors not generally considered capable of integration can do so at low frequencies. Integration of DNA might be desired or tolerated in target cells but should be minimised in non-target cells and is of particular concern for gonadal tissue where, although unlikely, there exists the potential for modifying the germline.

Integration events can result in insertional mutagenesis or genetic rearrangements that interrupt, induce or otherwise modify gene structure and/or expression. In addition, newer gene therapy technologies have resulted in vectors with higher titres, increased transduction efficiencies, and broader tropism, adding to the existing concerns regarding the risk of inadvertent germline integration. Regulatory authorities represented at the ICH agree that based on current scientific, ethical, and legal arguments gene therapy trials that are intended to allow direct germline integration should not be conducted. The regions also agree that the risk of inadvertent germline integration should be minimised because of the potential for subsequent transmission of vector DNA to progeny.

This document identifies general principles for investigating and addressing risks for inadvertent germline integration and provides considerations to minimise this potential risk in humans enrolled in clinical trials. This document applies to gene therapy vectors and could also apply to oncolytic viruses.

2. Risk factors for inadvertent germline integration of gene therapy vectors

The risk of inadvertent germline integration is based on a number of factors including vector type, dose, route, and site of administration; thus a science-based and case-by-case approach should be used in assessing this risk.

2.1. Vectors

The relative risk of germline integration associated with each vector category is generally based on the biodistribution profile, the replication capacity, and the integration potential for each vector type.

Vectors used in gene therapy research and clinical trials include, but are not limited to:

- adeno-associated viral (AAV) vectors
- adenoviral vectors
- gamma-retroviral vectors
- herpes simplex-1 viral (HSV-1) vectors
- lentiviral vectors
- paramyxoviral vectors
- plasmid vectors
- pox viral vectors

The ability of a vector to integrate into host cell chromosomes is a key factor in assessing the risk of germline integration. The following three categories list in descending order the relative ability of gene therapy vectors to integrate:

(i) Vectors that enter into the cell nucleus and that carry integration machinery
(ii) Vectors that enter into the cell nucleus, without integration machinery
(iii) Vectors that are unable to enter the target cell nucleus and remain cytoplasmic

For example, gamma-retroviral and lentiviral vectors have viral enzymes (e.g. integrase) that allow the vector to integrate. Therefore, these vectors are considered to have an increased risk of inadvertent germline integration. .

Additional properties to consider in risk assessment include replication competence and cell host range (tropism). Modification or pseudotyping of a viral vector can change the virus's natural tropism allowing dissemination to gonadal tissue, while replication competence might affect overall exposure.

2.2. Dose and route of administration

High doses might contribute to a higher statistical risk of gonadal exposure by the vector or vector components. Intravenous administration of gene therapy vectors could carry greater risk due to the increased potential for haematogenous spread to the gonads. Conversely, replication incompetent gene therapy vectors delivered by *ex vivo* transduction would represent a minimal risk of germline integration.

3. Non-clinical studies

3.1 General considerations

The clinical development of a gene therapy vector involves evaluation of both non-clinical and clinical safety information. Non-clinical studies are important in establishing safety and proof-of-concept for any product intended for clinical use. These studies also help define the risk:benefit ratio of the product for the intended clinical population. The design of non-clinical studies for biotechnology-derived pharmaceutical products is addressed in ICH S6. Although gene therapy vectors are specifically excluded from the scope of ICH S6, general principles described in this guideline could be relevant to this product class.

3.2 Biodistribution studies

Biodistribution studies in animals address vector dissemination to target and non-target organs and should be conducted with the product that is intended for use in clinical trials. Such studies should include analyses of gonads (testes and ovaries) because dissemination to gonadal tissue is an indication of the potential for germline integration. Vector dissemination can be detected using an assay for nucleic acid sequences. It is also recommended that testing for the presence of vector sequences in animal organs and tissues be done by at least one sensitive assay such as quantitative polymerase chain reaction (Q-PCR).

Prior biodistribution studies of the vector containing other transgenes could be used to support early phase clinical development.

If the vector is not detected in gonadal tissue, then further germline integration studies might not be warranted. If the vector is present in the gonads, animals should be studied to assess whether the level of vector sequence falls below the assay's limit of detection at later time points (i.e., transient detection). A persistent detection of vector sequences in the gonads might warrant elucidation of whether germline cells are transduced. When possible, testing of

gonadal tissue should be performed to determine whether vector is present within germ cells or non-germline cells (e.g., Sertoli cells, Leydig cells, leukocytes). In male animals, this can be accomplished by analyzing sperm at different time points based on the duration of spermatogenesis. Persistent detection of vector limited to only non-germline cells and/or a transient detection in sperm, likely indicates that germline integration has not occurred. In female animals, the present interpretation is that if vector is detected in one oocyte, all oocytes are affected. In cases where a persistent detection of vector sequences is observed in oocytes or sperm, the situation should be discussed with your regulatory authority.

4. Patient Monitoring

If, based on the animal biodistribution studies, the gene therapy vector is found to be transiently detected in the gonads, assaying patient semen for presence of vector might be considered. It is recognised that if the patient population is sterile, or the patient has a severe disease condition with a short life expectancy, monitoring of semen samples might not be warranted.

Testing should preferably be done at various time points and extend over at least one cycle of spermatogenesis, which is approximately 64-74 days in man. If semen samples are positive past one cycle of spermatogenesis, testing should continue and the respective regulatory authorities should be notified.

Currently there are no non-invasive means to monitor women for germline integration; therefore, the risk assessment for female subjects might be exclusively based on non-clinical data.

In clinical trials, regardless of the outcome of non-clinical biodistribution data, contraceptive measures are usually recommended for the duration of the clinical trial.